

Supplementary Table S1. Reference bacterial strains and culture conditions

Strain	Source	Culture Medium	For CFU assay
<i>Escherichia coli</i> O111:B4	The Institute of Medical Science, The University of Tokyo	Brain heart infusion (BHI) broth (Nissui Pharmaceutical), aerobically	Pearl core plate count agar (Eiken), aerobically
<i>Staphylococcus aureus</i> FDA209P	Food and Drug Administration, USA	BHI broth, aerobically	Pearl core plate count agar (Eiken), aerobically
<i>Lactobacillus casei</i> TISTR 390	Department of Science and Technology, Thailand	MRS broth (EMD Millipore Corp.), anaerobically	MRS agar in an anaerobic chamber (Becton Dickinson and Co., Cockeysville, USA)
<i>Bifidobacterium longum</i> BB536	Morinaga Milk Industry, Japan	BL agar (Nissui Pharmaceutical Co., anaerobically	BL agar in an anaerobic chamber
<i>Bacteroides fragilis</i> JCM 11019(NCTC 9343)	Riken BioResource Center, Japan	BHI broth, anaerobically	BHI agar in an anaerobic chamber

Supplementary Table S2. The primers used for Real-Time PCR

Bacteria	Sequence (5'-3')		PCR reaction	Product size (bp)	Reference
All Bacteria	Forward	TCCTACGGGAGGCAGCAGT	60°C-20s	466	34
	Reverse	GGACTACCAGGGTATCTAATCCTGTT	& 72°C-20s		
<i>Bifidobacterium</i>	Forward	CTCCTGGAACGGGTGG	55°C-20s	549-563	35
	Reverse	GGTGTTCCTCCGATATCTACA	& 72°C-50s		
<i>Lactobacillus</i>	Forward	TGGAAACAGRTGCTAATACCG	60°C-31s	231-233	36
	Reverse	GTCCATTGTGGAAAGATCCC			
<i>Bacteroides</i>	Forward	ATAGCCTTCGAAAGRAAGAT	50°C-20s	501	35
	Reverse	CCAGTATCAACTGCAATTAA	& 72°C-30s		
<i>Escherichia coli</i>	Forward	GTTAATACCTTGCTCATGAA	60°C-31s	340	37
	Reverse	ACCAGGGTATCTAATCCTGTT			
<i>Staphylococcus</i>	Forward	ACGGTCTGCTCACTTATA	60°C-31s	257	38
	Reverse	TACACATATGTTCTCCCTAATAA			

Supplementary Table S3. Effects of demographic factors on bacteria counts, LPS-related biomarkers, and anti-bacterial substance antibody levels

	Age			Sex			Duration (months)			Smoking			Drinking					
	<65 (N=29)		≥65 (N=58)	P-value	Male (N=20)		Female (N=67)	P-value	<36 (N=6)		≥36 (N=81)	P-value	No (N=44)	Yes ^a (N=43)	P-value	No (N=23)	Yes ^b (N=64)	P-value
Total bacteria (log/g of feces)	11.80±0.10	11.85±0.06	0.715	11.82±0.09	11.84±0.06	0.576	12.04±0.26	11.82±0.05	0.167	11.83±0.08	11.84±0.06	0.625	11.85±0.10	11.83±0.06	0.897			
<i>Bifidobacterium</i>	9.22±0.17	9.01±0.13	0.463	9.18±0.22	9.05±0.12	0.632	9.49±0.17	9.05±0.11	0.265	9.19±0.12	8.97±0.17	0.291	9.07±0.23	9.08±0.12	0.725			
<i>Lactobacillus</i>	8.83±0.26	9.79±0.15	0.003*	10.1±0.26	9.29±0.16	0.013*	9.68±0.48	9.46±0.15	0.719	9.45±0.22	9.50±0.18	0.875	9.88±0.24	9.33±0.17	0.101			
<i>Bacteroides</i>	7.59±0.19	7.77±0.14	0.446	7.76±0.20	7.70±0.13	0.988	7.85±0.43	7.70±0.12	0.756	7.75±0.16	7.67±0.15	0.619	7.57±0.21	7.65±0.13	0.404			
<i>E. coli</i>	7.73±0.28	7.82±0.18	0.631	7.69±0.32	7.82±0.17	0.655	8.46±0.53	7.74±0.15	0.203	8.02±0.21	7.55±0.20	0.094	7.77±0.30	7.80±0.17	0.915			
<i>Staphylococcus</i>	7.33±0.17	7.63±0.13	0.172	7.46±0.21	7.54±0.12	0.848	7.29±0.37	7.54±0.11	0.558	7.61±0.15	7.43±0.15	0.476	7.39±0.24	7.57±0.11	0.421			
Fecal LPS (ng/g)	29.74±1.9	20.1±3.17	0.477	39.2±15.2	18.6±2.91	0.617	16.3±3.69	23.8±4.50	0.621	20.0±4.16	26.7±7.37	0.750	26.7±11.7	22.4±3.93	0.441			
Serum LPS (pg/ml)	3.90±0.22	3.93±0.14	0.698	3.80±0.24	3.95±0.13	0.648	3.76±0.48	3.93±0.12	0.756	4.03±0.17	3.80±0.16	0.248	3.99±0.14	3.53				
LBP (μg/ml)	24.2±2.18	29.7±1.99	0.179	30.6±3.06	27.0±1.77	0.256	35.7±7.07	27.3±1.56	0.175	26.1±1.98	29.7±2.34	0.408	27.4±2.91	28.0±1.81	0.806			
ENC (ng/ml)	14.03±0.07	14.07±0.06	0.275	13.99±0.09	14.09±0.05	0.325	14.27±0.16	14.04±0.05	0.476	14.05±0.08	14.06±0.06	0.541	14.16±0.09	14.02±0.05	0.116			
Anti <i>E. coli</i> -LPS IgG (kU/ml)	4.96±0.84	7.46±0.98	0.162	5.06±1.44	7.09±0.83	0.041*	9.04±3.73	6.44±0.73	0.466	7.94±1.12	5.28±0.86	0.019*	6.15±1.01	6.79±0.91	0.973			
Anti <i>E. coli</i> -LPS IgA (kU/ml)	3.92±0.81	4.61±0.64	0.390	4.31±1.06	4.40±0.58	0.576	5.92±3.01	4.27±0.50	0.874	5.07±0.71	3.67±0.71	0.012*	4.90±0.93	4.19±0.60	0.400			
Anti Pg-LPS IgG (kU/ml)	82.3±29.4	63.0±12.3	0.466	44.2±8.26	77.0±16.3	0.542	44.9±16.0	71.2±13.6	0.913	69.6±19.7	69.2±16.2	0.622	56.1±12.8	74.2±6.7	0.751			
Anti Pg-LPS IgA (kU/ml)	3.07±1.08	2.94±0.85	0.611	1.48±0.34	3.41±0.36	0.936	0.62±0.20	3.14±0.71	0.111	2.67±0.80	3.27±1.09	0.249	1.20±0.27	3.60±0.89	0.476			
Anti PG-PS IgG (kU/ml)	207.1±33.4	239.0±19.1	0.106	221.1±31.0	230.5±20.0	0.844	264.2±78.5	225.7±17.3	0.633	220.9±23.5	236.1±24.6	0.533	231.7±29.0	227.2±20.6	0.683			
Anti PG-PS IgA (kU/ml)	43.1±12.1	57.9±10.3	0.110	48.6±14.5	54.2±6.42	0.690	42.1±24.2	53.7±8.38	0.519	44.9±8.88	61.2±13.3	0.384	55.8±14.2	51.9±9.60	0.319			

Patients enrolled in this study were divided into two groups depending on age, sex, disease duration, smoking, and drinking.

Effects of demographic factors on the intestinal commensal bacteria, LPS-related biomarkers, and anti bacterial antibodies levels between two groups were compared using Wilcoxon rank sum test.

Data were shown as mean±standard error and P-value. Significant difference: *P<0.05.

LPS: Lipopolysaccharide, LBP: LPS-binding protein, ENC: endotoxin neutralizing capacity, *E. coli*-LPS: LPS from *E. coli*, Pg-LPS: LPS from *Porphymonas gingivalis*, and PG-PS: peptidoglycan polysaccharide.

a: including patients who have a history of smoking b: sum of patients who drink on a regular basis.

Supplementary Table S4. Effects of treatments on bacteria counts, LPS-related biomarkers, and anti-bacterial substance antibody levels

	MTX (mg/week)		MTX (mg/week) + Other csDMARDs		PSL (mg/day)		
	Non-treated (N=32)	Treated (N=55)	MTX alone (N=17)	MTX with other csDMARDs (N=38)	Non-treated (N=43)	Treated (N=44)	
	MTX: 0	MTX: 7.8±0.3	MTX: 8.4±0.6	MTX: 7.6±0.4	PSL: 0	PSL: 4.0±0.2	
Total bacteria (log/g of feces)	11.65±0.09	11.94±0.06	0.004*	11.99±0.10	11.90±0.08	0.629	11.84±0.07
<i>Bifidobacterium</i>	8.98±0.18	9.14±0.13	0.386	9.18±0.30	9.05±0.15	0.275	9.11±0.13
<i>Lactobacillus</i>	9.29±0.26	9.57±0.17	0.449	9.63±0.32	9.57±0.20	0.978	9.32±0.21
<i>Bacteroides</i>	7.66±0.19	7.74±0.14	0.669	7.84±0.26	7.68±0.16	0.554	7.72±0.16
<i>E. coli</i>	7.88±0.24	7.74±0.19	0.604	7.75±0.34	7.70±0.23	0.971	7.83±0.22
<i>Staphylococcus</i>	7.26±0.15	7.67±0.14	0.087	7.56±0.33	7.74±0.14	0.334	7.46±0.15
Fecal LPS (μg/g)	30.8±9.99	18.9±3.18	0.993	18.6±4.02	19.1±7.88	0.472	22.6±4.45
Serum LPS (pg/ml)	4.30±0.20	3.69±0.14	0.018*	3.43±0.20	3.81±0.17	0.201	3.82±0.17
LBP (μg/ml)	30.9±2.76	26.1±1.79	0.097	26.5±3.55	25.9±2.07	0.993	28.1±2.49
ENC (ng/ml)	14.12±0.06	14.02±0.07	0.733	14.15±0.08	13.96±0.09	0.563	14.05±0.07
Anti <i>E. coli</i> -LPS IgG (kU/ml)	5.81±0.90	7.12±1.02	0.401	7.90±2.48	6.67±0.97	1.000	6.88±0.89
Anti <i>E. coli</i> -LPS IgA (kU/ml)	4.44±0.84	4.35±0.64	0.864	5.89±1.48	3.64±0.61	0.187	4.69±0.69
Anti Pg-LPS IgG (kU/ml)	61.1±21.4	74.3±15.9	0.035*	61.4±14.6	80.0±21.9	0.629	89.8±24.6
Anti Pg-LPS IgA (kU/ml)	3.77±1.31	2.50±0.73	0.005*	1.73±0.72	2.84±1.00	0.956	3.83±1.15
Anti PG-PS IgG (kU/ml)	200.3±20.9	245.6±23.8	0.269	232.7±46.2	249.4±27.6	0.387	237.6±24.8
Anti PG-PS IgA (kU/ml)	53.3±10.8	52.7±11.0	0.318	75.9±27.8	42.1±9.65	0.358	48.1±7.06
							57.7±14.2
							0.314

The 87 patients enrolled in this study were divided into two groups based on MTX, csDMARDs, and steroid treatment (Prednisolone: PSL). Thirty-two patients were not treated with MTX during this study period, whereas 55 patients were treated with MTX alone (N=17) or a combination of MTX and other csDMARDs (N=38). Other csDMARDs are BUC (15 cases), TAC (7), SASP (4), TAC+SASP (1), BUC + SASP (4), injectable Gold (3), Actarit (1), IGU (1), LEF (1), and injectable Gold+ BUC (1). The MTX nontreated 32 patients were treated with SASP (8), BUC (7), LEF (4), TAC (3), TAC + BUC (3), Mino (1) and none (5), respectively.

Effects of treatments on the intestinal commensal bacteria, LPS-related biomarkers, and anti Pg-LPS antibody levels between the two groups were compared using Wilcoxon rank sum test. Data were shown as mean±standard error and P-value. Significant difference: *P< 0.05.

LPS: Lipopolysaccharide, LBP: LPS-binding protein, ENC: endotoxin neutralizing capacity, *E. coli*-LPS: LPS from *E. coli*, Pg-LPS: *Porphyromonas gingivalis* LPS, PG-PS: peptidoglycan polysaccharide, MTX: methotrexate, csDMARDs: conventional synthetic disease-modifying anti-rheumatic drugs, BUC: Bucillamine, SASP: Sulfasalazine, LEF: Leflunomide, TAC: Tacrolimus, IGU: Iguratimod, Mino: Minomycin, and AUR: Auranofin.